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(54) COMPOSITION FOR PREVENTING AND/OR TREATING OPHTHALMOPATHY (EXCLUDING OPHTHALMOPATHY CAUSED BY DRY EYE) CAUSED BY APOPTOSIS

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain a composition for preventing and/or treating ophthalmopathy (excluding ophthalmopathy caused by dry eye) caused by apoptosis.
SOLUTION: This composition for preventing and/or treating the ophthalmopathy (excluding ophthalmopathy caused by dry eye) caused by apoptosis contains 3-hydroxybutyric acid and/or its salt as an active ingredient. Further, the composition for preventing and/or treating the ophthalmopathy (excluding the ophthalmopathy caused by the dry eye) caused by the apoptosis, especially keratoconjunctivitis, contains the above active ingredient.

CLAIMS

[Claim(s)]
[Claim 1]A constituent for prevention of an obstacle (except for an obstacle by dry eye) of an eye by apoptosis, and/or a therapy which contains 3-hydroxybutyric acid and/or its salts as an active principle.
[Claim 2]The constituent according to claim 1, wherein 3-hydroxybutyric acid is D-object.
[Claim 3]The constituent according to claim 1 or 2 which is at least one sort chosen from a group which salts of 3-hydroxybutyric acid become from sodium salt, potassium salt, L-lysine salt, an L-histidine salt, and an L-arginine salt.
[Claim 4]The constituent according to any one of claims 1 to 3 whose concentration of 3-hydroxybutyric acid and/or its salts is 0.1 - 1000 mmol/L.
[Claim 5]The constituent according to any one of claims 1 to 4 whose concentration of 3-hydroxybutyric acid and/or its salts is 1 - 150 mmol/L.
[Claim 6]The constituent according to any one of claims 1 to 5 whose obstacle (except for an obstacle by dry eye) of an eye by apoptosis is an obstacle of an angle conjunctiva.

NOTICES *

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates to the constituent for prevention of the obstacle (except for the obstacle by dry eye) of the eye by apoptosis, and/or a therapy which contains 3-hydroxybutyric acid and/or its salts as an active principle. the obstacle (except for the obstacle by dry eye) of the eye by apoptosis which contains the above-mentioned active principle in more detail -- it is especially related with the constituent for prevention of the obstacle of an angle conjunctiva, and/or a therapy.
[0002]
[Description of the Prior Art]Apoptosis (apoptosis) is cell death which happens when the cell disintegration program controlled by the gene of the cell moves. The feature of this apoptosis has cell reduction, chromatin condensation, fragmentation of chromatin DNA, an appearance, cell fragmentation of an apoptotic body, etc., and it is thought that it is a gestalt of the active cell death which advances to the inside of a short time.
[0003]In recent years, many researches on this apoptosis are made and are being

gradually solved about that mechanism. Activation of caspase which is a part of especially the mechanism has played the important role about derivation of apoptosis. It is thought before long that the caspase 3 has played the important role especially. Therefore, it is very important to control caspase 3 activity, in order to control apoptosis.
[0004]Although apoptosis is an indispensable phenomenon in the generating process of a living thing, maintenance of homeostasis, etc., Being derived by being derived by physical stress, such as spontaneous generation, lack of various-causes children, such as a nutritional factor and a growth factor, and radiation, etc., in addition removing a blood serum from a culture medium, for example in a cell culture is also known.
[0005]Now, relevance with apoptosis is suggested in diseases, such as cancer cell death by the reduction and the various anticancer agents of a lymphocyte in an acquired immunodeficiency syndrome, Parkinson's disease, dementia, and ischemic neuronal death.
[0006]It is thought that apoptosis is participating in various diseases besides above mentioned various diseases. As an obstacle about an eye, the retinopathy by the ischemia and reperfusion, glaucoma, optic nerve cutting, amotio retinae, retina degeneration, a cataract, etc. are mentioned among the obstacles it is suggested that apoptosis is involving saying, for example. It is suggested in recent years that apoptosis is involving also in the obstacle of an angle conjunctiva. It is suggested that apoptosis is involving in the obstacle by herpesvirus, Acanthamoeba, platelet activating factor, a benzalkonium chloride, ultraviolet rays, etc., the cause of a disease of the keratoconus, and the cornea damage to after excimer laser irradiation especially.
[0007]Some of drugs which treat the retinopathy, the glaucoma, and the cataract it is supposed that apoptosis is involving among the obstacles about the above mentioned eye, or its method is indicated. For example, the retina disease treating agent containing alpha-phenyl-t-butyl-nitrone and its derivative. The retinopathy relevant to the cell death or the aging process of a retina nerve which may produce the obstacle (for example, glaucoma) which (for example, patent-documents 1 reference) and intraocular pressure increase. The apoptotic-cell-death inhibitor (for example, refer to patent documents 2) which prevents (for example, age-related macular degeneration), and the method (for example, refer to patent documents 3) of barring the cell regeneration after a cataract operation are indicated, respectively.
[0008]However, it is not known that 3-hydroxybutyric acid which is an active principle of this invention is effective to the prevention and/or the therapy of the obstacle (except for the obstacle by dry eye) of an eye by apoptosis.
[0009]
[Patent documents 1] JP,9-278652,A[Patent documents 2] The ** table No. 519358 [2001 to] gazette [Patent documents 3] The Patent Publication Heisei No. 508575 [ten to] gazette [0010]
[Problem(s) to be Solved by the Invention]The purpose of this invention is to provide the constituent for prevention of the obstacle (except for the obstacle by dry eye) of the eye by apoptosis, and/or a therapy which contains 3-hydroxybutyric acid and/or its salts as an active principle. It is in the obstacle (except for the obstacle by dry eye) of the eye containing the above-mentioned active principle by apoptosis, and providing the constituent for prevention of the obstacle of an angle conjunctiva, and/or a therapy especially in more detail.

[0011]
[Means for Solving the Problem]According to this invention, the above-mentioned purpose and an advantage of this invention are attained by the following.
(1) A constituent for prevention of an obstacle (except for an obstacle by dry eye) of an eye by apoptosis, and/or a therapy which contains 3-hydroxybutyric acid and/or its salts as an active principle.
(2) A constituent of the above-mentioned (1) statement, wherein 3-hydroxybutyric acid is D-object.
(3) A constituent of the above (1) which is at least one sort chosen from a group which salts of 3-hydroxybutyric acid become from sodium salt, potassium salt, L-lysine salt, an L-histidine salt, and an L-arginine salt, or the above-mentioned (2) statement.
(4) A constituent given in either the above (1) whose concentration of 3-hydroxybutyric acid and/or its salts is 0.1 - 1000 mmol/L - the above (3).
(5) A constituent given in either the above (1) whose concentration of 3-hydroxybutyric acid and/or its salts is 1 - 150 mmol/L - the above (4).
(6) A constituent given in either the above (1) whose obstacle (except for an obstacle by dry eye) of an eye by apoptosis is an obstacle of an angle conjunctiva - the above (5).
[0012]
[Embodiment of the Invention]In this invention, the 3-hydroxybutyric acid used as an active principle, Are known as a biogenic substance, and it is generated when fatty acid oxidizes by liver, Being used as an energy source in a peripheral organization is known (Ikuo Yamashina editorial supervision, "Lehninger's new chemicals (above)", the 2nd edition, Hirokawa Publishing, April 15, 1993, p625 -626 reference). D-object, D, and L-racemate and L-object are known about the C3 place configuration of the chemical constitution formula of 3-hydroxybutyric acid. In this invention, although these [all] can be used, D-object is [among these] the most preferred on prevention of the obstacle (except for the obstacle by dry eye) of the eye by apoptosis, and/or a therapy. The salts of 3-hydroxybutyric acid are preferred and at least one sort is suitably chosen from the group which consists of sodium salt, potassium salt, L-lysine salt, an L-histidine salt, and an L-arginine salt. these 3-hydroxybutyric acid and/or its salts are independent suitably -- it is -- two or more kinds can be used together. As for the concentration of 3-hydroxybutyric acid in the constituent of this invention, and/or its salts, it is preferred that there is especially 0.5 to 500 mmol/L in the range of 1 - 150 mmol/L further more preferably 0.1 to 1000 mmol/L according to a patient's age and condition, and its use.
[0013]In this invention, 3-hydroxybutyric acid which is an active principle is effective to the prevention and/or the therapy of the obstacle (except for the obstacle by dry eye) of an eye by apoptosis. the obstacle (except for the obstacle by dry eye) of the eye according [the constituent of this invention which contains the above-mentioned active principle in more detail] to apoptosis -- it is especially effective to prevention and/or the therapy of the obstacle of an angle conjunctiva. As an obstacle of the eye by the apoptosis in which the constituent of this invention is especially effective, the cornea damage to after the obstacle by herpesvirus, Acanthamoeba, platelet activating factor, a benzalkonium chloride, ultraviolet rays, etc., the keratoconus, and excimer laser irradiation, etc. are mentioned.
[0014]the constituent of this invention -- taking orally ---like (a tablet, granulation, etc.) -- or -- being parenteral (ophthalmic solutions, drops, etc.) -- it is used suitably. As a

gestalt of the pharmaceutical preparation which consists of a constituent of this invention, ophthalmic solutions, drops, injections, ophthalmic ointments, a tablet, granulation, powder medicine, a capsule, etc. are mentioned, and all can be suitably prepared by a publicly known method, for example. The isotonizing agent, the buffer, stabilizing agent, antiseptic which are usually used for these pharmaceutical preparation, A viscous agent, a pH adjuster, an excipient, a binding material, a dispersing agent, a resorption accelerator, a surface-active agent, A solubilizing agent, an emulsifier, a moisturizer, colorant, perfume, an organic solvent (for example, alcohols, oil), silicone, a polymer, a solid fat substance (for example, waxes), etc. can be contained, and according to a use, it can choose suitably and can prepare.

[0015]When carrying out local administration of the constituent of this invention especially to an eye also in the gestalt of the above mentioned pharmaceutical preparation, it is preferred to consider it as ophthalmic solutions or ophthalmic ointments, and it is still more preferred to consider it as ophthalmic solutions.

[0016]For example, when using the constituent of this invention as ophthalmic solutions, an isotonizing agent, a buffer, a stabilizing agent, a viscous agent, a pH adjuster, etc. can be contained if needed the stability of ophthalmic solutions, and for the purpose of putting and obtaining the goodness of a feeling. these ingredients are independent -- it is -- two or more kinds can be contained suitably, and it is more preferred when it is many.

[0017]As an isotonizing agent which can be contained when using the constituent of this invention as ophthalmic solutions, especially if generally used as ophthalmic solutions, it will not interfere. When using the constituent of this invention as ophthalmic solutions, an isotonizing agent can be contained in order to prepare the osmotic pressure of these ophthalmic solutions. As such an isotonizing agent, for example Sodium chloride, potassium chloride, Sugar, such as the alkali or the mineral salt like alkaline earth metal salt which consists of a calcium chloride, a magnesium chloride, magnesium sulfate, etc. and glucose, mannitol, sorbitol, xylitol, and dextran, etc. are mentioned. these are independent -- it is -- two or more kinds can be used together. It is more preferred that it is 0.01 - 3 w/v% preferably as concentration of these isotonizing agents that it is 0.001 - 5 w/v%.

[0018]As a buffer which can be contained when using the constituent of this invention as ophthalmic solutions, especially if generally used as ophthalmic solutions, it will not interfere. When using the constituent of this invention as ophthalmic solutions, a buffer can be contained in order to stabilize pH of these ophthalmic solutions. As such a buffer, for example Phosphoric acid 1 hydrogen disodium, a sodium dihydrogenphosphate, The tris system buffer like the boric acid system buffer, the tris aminomethane and dilute hydrochloric acid and tris malate like a phosphoric acid system buffer, boric acid, and the sodium borate like phosphoric acid 1 hydrogen dipotassium and potassium dihydrogen phosphate, and rare caustic soda liquid, etc. are mentioned. these are independent -- it is -- two or more kinds can be used together. As concentration of these buffers, it is preferred that it is 0.001 - 5 w/v%, and it is more preferred that it is 0.01 - 1 w/v%.

[0019]As a stabilizing agent which can be contained when using the constituent of this invention as ophthalmic solutions, especially if generally used as ophthalmic solutions, it will not interfere. When using the constituent of this invention as ophthalmic solutions, a stabilizing agent can be contained in order to stabilize the active principle of these ophthalmic solutions. As such a stabilizing agent, disodium ethylenediaminetetraacetate,

citrate, citrate, etc. are mentioned, for example. these are independent -- it is -- two or more kinds can be used together. As concentration of these stabilizing agents, it is preferred that it is 0.001 - 5 w/v%, and it is more preferred that it is 0.01 - 1 w/v%.
[0020]As a viscous agent which can be contained when using the constituent of this invention as ophthalmic solutions, especially if generally used as ophthalmic solutions, it will not interfere. When using the constituent of this invention as ophthalmic solutions, a viscous agent can be contained in order to prepare the viscosity of these ophthalmic solutions. As such a viscous agent, for example Glycerin, ethylene glycol, Polyols, such as propylene glycol, a polyethylene glycol, and polyvinyl alcohol. Trehalose, sucrose, carboxymethyl cellulose, hydroxyethyl cellulose, Polycarboxylic acid/salts, such as sugar, such as hydroxypropylmethylcellulose and cyclodextrin, a carboxyvinyl polymer, citrate, and edetate, are mentioned, in addition hyaluronate, povidone, etc. can be contained. these are independent -- it is -- two or more kinds can be used together. As concentration of these consistency agent, it is preferred that it is 0.001 - 10 w/v%, and it is more preferred that it is 0.01 - 5 w/v%.
[0021]As a pH adjuster which can be contained when using the constituent of this invention as ophthalmic solutions, especially if generally used as ophthalmic solutions, it will not interfere. When using the constituent of this invention as ophthalmic solutions, a pH adjuster can be contained in order to prepare pH of these ophthalmic solutions. As such a pH adjuster, chloride, citrate or its salt, boric acid or its salt, phosphoric acid or its salt, acetic acid or its salt, tartaric acid or its salt, sodium hydroxide, a potassium hydrate, etc. are mentioned, for example. these are independent -- it is -- two or more kinds can be used together. The constituent of this invention adds a proper quantity of these pH adjusters, and prepares them to the target pH.
[0022]On the other hand, when using the constituent of this invention as ophthalmic solutions, an antiseptic can also be contained in order to give an antiseptic effect to these ophthalmic solutions. In this case, as for a benzalkonium chloride, since there is a possibility of causing apoptosis, it is not preferred [as an antiseptic which can be contained, especially if generally used as ophthalmic solutions, it will not interfere, but] to use it with the constituent of this invention. As an antiseptic which can be contained in the constituent of this invention, ethylparaben, butylparaben, benzethonium chloride, chlorhexidine glyconate, citrate, boric acid, etc. are mentioned, for example. these are independent -- it is -- two or more kinds can be used together. As concentration of these antiseptics, it is preferred that it is 0.0001 - 0.1 w/v%, and it is more preferred that it is 0.001 - 0.05 w/v%.
[0023]However, it is more desirable not to contain an antiseptic, since the obstacle of an eye may get worse further by applying eyewash in the ophthalmic solutions containing an antiseptic when having produced the obstacle of the eye generally. It is more desirable not to contain an antiseptic, since it may produce an obstacle in an eye with an antiseptic similarly when you need frequent instillation even if it is a case where the obstacle is not produced in an eye. When it does not contain an antiseptic, it is preferred to fill up what is called the container of the type which throws away the ophthalmic solutions of this invention using one use, and a "DISUPO container."
[0024]When using the constituent of this invention as ophthalmic solutions, the antihistamine generally used as an agent for ophthalmology, vasoconstrictor, resolution and an astringent, lubricant, amino acid, an antiallergic agent, vitamins, sulfa drugs, local

anesthetic, a miotic, etc. can also be contained as other ingredients. When these ingredients are contained, according to a patient's age, condition, etc., it is necessary to consider it as the ingredient and concentration for which the effect of 3-hydroxybutyric acid which is an active principle is not affected.
[0025]When using the constituent of this invention as ophthalmic solutions, it is preferred to set pH range to pH 5-8 combining various ingredients. Since an eye stimulus and an ophthalmopathy may be produced, it is not desirable in pH five or less acidity or pH eight or more alkaline regions. About osmotic pressure, it is preferred to consider it as the range of 150 - 700mOsm. to an eye, so that safely.
[0026]When using the constituent of this invention as ophthalmic solutions, if it is within the limits supplied ophthalmologically, a dose in particular is not restricted, and it is usually preferred to prescribe 1-3 drops per time for the patient about several times per day.
[0027]
[Example]Hereafter, although an example and a comparative example explain this invention concretely, this invention is not limited to these.
[0028]In order to investigate the effect over the cell activity of the Homo sapiens conjunctival epithelial cell which performed the blood serum removal stimulus of example 1 blood serum and D-3-hydroxybutyric acid (it omits the following HBA), it experimented in accordance with the following method.
** 48well plate -- the Homo sapiens conjunctival epithelial cell (CCL20.2) -- every [$5x10^{4}$/well] -- seeding was carried out and it cultivated for two days 10% with blood serum content culture medium (a blood serum is a product made by Invitrogen).
** Culture medium was removed after the end of culture, phosphoric acid buffer solution (it abbreviates to PBS below) washed twice, and it replaced by each culture medium containing the blood serum or HBA of various concentration, and cultivated for 24 hours. The osmotic pressure of each culture medium was altogether prepared to 470mOsm. using sodium chloride except control.
** Each culture medium was removed after the end of culture, and the 10% ARAMA blue content culture medium per well was added. This ARAMA blue content culture medium was made to contain a blood serum 10%.
** every [in / with a fluorescence plate leader (made by Applied Bio Systems) / excited wavelengths of 530 nm, and the fluorescence wavelength of 580 nm] -- the fluorescence intensity (fluorescence intensity 1) of well was measured.
** After that, it cultivated within CO_2 incubator for 30 minutes, and fluorescence intensity (fluorescence intensity 2) was measured again.
** From the fluorescence intensity 2, the increase of stock of fluorescence intensity was computed by having deducted the fluorescence intensity 1, and this value was made into cell activity. About control, the cell activity after the first culture during two days was measured, and the value was considered as control. In all the examples, culture medium used 199 culture media (made by NISSUI PHARMACEUTICAL CO., LTD.). In this example, 4well was used per monograph affairs other than control. The result performed in accordance with the above-mentioned method is shown in drawing 1. The cell activity of each culture medium containing the blood serum or HBA of various concentration was shown as a relative activity value to the cell activity of control.
[0029]From the result of drawing 1, by removing a blood serum, the activity of the cell

fell substantially and fell to about 50% of control. Under [HBA controls this cell activity fall on a concentration dependence target, 80 mmol/L HBA shows about 70% of cell activity of control and this is equivalent to the value of 5% of a blood serum]. This result showed HBA controlling the cell activity fall by blood serum removal, and having depressor effect almost equivalent to a blood serum.
[0030]It was investigated whether it would be because the depressor effect of the cell activity fall by example 2HBA controls reduction in a cell number for whether the fall of the activity of a cell itself is controlled. Using 10% blood serum content culture medium, 80 mmol/L HBA content culture medium, and blood serum non-containing culture medium, as 3, 6, 12, and 24 hours, cell activity was measured and each culture medium processing time was simultaneously measured also about the cell number by the method shown in Example 1. $6.6x10^5$ individual seeding of the cell was carried out to the 25-cm^2 flask. The result about cell activity is shown in drawing 2, and the result about a cell number is shown in drawing 3.
[0031]The result of drawing 2 and drawing 3 showed that a cell activity fall originated in reduction in a cell number from cell activity falling with reduction in a cell number. That is, it turned out that cell death has happened. In 80 mmol/L HBA content culture medium, since cell death was controlled compared with blood serum non-containing culture medium, it turned out that HBA has the outstanding cell death depressor effect to the cell death by blood serum removal. It is known that the cell death by blood serum removal will be based on apoptosis.
It turned out that HBA has an effect which controls the cell death by apoptosis.

[0032]The activity of the caspase which is participating in derivation of example 3 apoptosis was investigated using a blood serum and HBA. This example was performed by the following method.
** $2x10^6$ individual seeding of the Homo sapiens conjunctival epithelial cell (CCL20.2) was carried out to the 75-cm^2 flask, and it cultivated for two days 10% with blood serum content culture medium (a blood serum is a product made by Invitrogen).
** Culture medium was removed after the end of culture, and it washed twice in PBS, and it replaced by each culture medium containing a blood serum or HBA, and cultivated. The osmotic pressure of each culture medium was altogether adjusted to 470mOsm. using sodium chloride except control.
** After removing culture medium after processing each culture processing time as 3, 6, 12, and 24 hours, and washing twice in PBS, trypsinization recovered the cell of each flask and the cell was settled by centrifugal separation.
** After adding PBS cooled on ice and making a cell re-float, the cell was settled by centrifugal separation.
Operation of **** was repeated once again.
** After settling a cell by centrifugal separation and removing PBS, it was made to dissolve in a dissolution buffer (50 mmol/L HEPES, 1 mmol/L DTT, 0.1 mmol/LEDTA, 0.1%CHAPS, 0.1 mmol/L PMSF, pH 7.4).
** It extracted 1micro every L supernatant liquid after centrifugal separation, and the protein concentration in the extracted supernatant liquid was measured by the BCA micro method.
** To 96well plate, they are supernatant liquid protein and an assay buffer (50 mmol/L

HEPES). 100 mmol/L NaCl, 10 mmol/L DTT, 1 mmol/L EDTA, 10% glycerol, 0.1%CHAPS, 0.1 mmol/L PMSF, pH 7.4 and a 100micromol/L caspase substrate [Ac-Asp-Glu-Val-Asp-AFC (made by Enzyme Systems Products)] were prepared so that it might become the total capacity L of 100micro. Here, caspase acts on this substrate, AFC is separated from this substrate, and that separated AFC shows a fluorescence. Then, after incubating this solution at 37 ** for 1 hour, the fluorescence intensity of each well in excited wavelengths of 400 nm and the fluorescence wavelength of 508 nm was measured with the fluorescence plate leader. Fluorescence intensity was similarly measured only about the AFC standard and substrate which are fluorescent substances.
** What deducted the value of only a substrate from the value of each well was made into the value of each sample, each sample AFC generated amount was computed from the AFC standard straight line, and the caspase activation per protein volume was computed. The result performed in accordance with the above-mentioned method is shown in drawing 4.
[0033]From the result shown in drawing 4, 80 mmol/L HBA content culture medium was compared with blood serum non-containing culture medium, and the caspase activation depressor effect outstanding 3 hours, 6 hours, and 12 hours afterward was shown. The caspase activation depressor effect which was excellent 6 hours and 12 hours afterward to blood serum content culture medium was shown, and the almost equivalent effect was shown 24 hours afterward. These showed that HBA controlled the activity of the caspase which participates in derivation of apoptosis.
[0034]In example 4 Example 3, when observed in accordance with the gestalt of a cell, it is 3 hours after culture and omission of the cell by apoptosis, etc. were already accepted. So, in accordance with the method shown in Example 3, the caspase activation before omission of a cell was measured for the purpose of investigation of the caspase activation depressor effect by HBA. Each processing time was performed as 0 minute, 15 minutes, 30 minutes, 45 minutes, and 60 minutes. The result is shown in drawing 5.
[0035]From the result shown in drawing 5, 80 mmol/L HBA content culture medium showed the outstanding caspase activation depressor effect in main culture processing time as compared with blood serum content culture medium, and blood serum both non-containing culture medium. These showed that HBA controlled the activity of the caspase which participates in derivation of apoptosis for a short time.
[0036]The chromatin condensation which is participating in derivation of example 5 apoptosis was investigated using a blood serum and HBA. This example was performed by the following method.
** The Homo sapiens conjunctival epithelial cell (CCL20.2) cultured by the method shown in Example 1 was replaced by 10% blood serum content culture medium, 80 mmol/L HBA content culture medium, and blood serum non-containing culture medium, and each culture medium processing time was processed as 1, 3, and 6 hours. 80 mmol/L HBA content culture medium, and blood serum non-containing culture medium adjusted osmotic pressure to 470mOsm. using sodium chloride.
** Each culture medium was removed after processing and the solution which dissolved Hoechst33342 (it omits the following Ho) coloring matter in PBS, and was made into 10microg/mL was incubated for 30 minutes within 100microL, in addition CO_2 incubator per well.
** every [in / solutions are collected and / with a fluorescence plate leader (made by

Applied Bio Systems) / excited wavelengths of 360 nm, and the fluorescence wavelength of 450 nm] -- the fluorescence intensity of well was measured.
** The cultured cell was washed by PBS after measurement, and the solution which dissolved neutral red (it omits the following Nr) in 199 culture media, and was made into 0.005% was incubated within 200microL, in addition CO_2 incubator per well for 3 hours.
** After removing the stain solution and washing a cultured cell twice in PBS, it processed with the ethanol solution 1% acetic acid and 50% for 15 minutes at the room temperature, and Nr was extracted.
** every [in / for an extract / with a fluorescence plate leader (made by Applied Bio Systems) / excited wavelengths of 535 nm, and the fluorescence wavelength of 600 nm] -- the fluorescence intensity of well was measured.
** What lengthened the fluorescence intensity (value which measured only Ho liquid only with Nr liquid) of each solution for which it asked as blank from the fluorescence intensity of each solution (Ho liquid and Nr liquid) was made into Ho value and Nr value.
** Ho relative value and Nr relative value of the each object of the treatment group at the time of setting Ho value of the group untaken a measure and the average value of Nr value to 1.0 were computed.
** Ho relative value was broken by Nr relative value, and Ho/Nr ratio of the each object was calculated.
The result performed in accordance with the above-mentioned method is shown in drawing 6. Here, a Ho/Nr value shows the degree of chromatin condensation per unit cell. By the group untaken a measure, if a Ho/Nr value is set to 1.0 and a value becomes large from 1.0, it is shown that apoptosis has happened in proportion to it. When a value becomes small from 1.0, it is shown that necrosis (necrosis) has happened.
[0037]The cell processed from the result shown in drawing 6 with 80 mmol/L HBA content culture medium and 10% blood serum content culture medium, Since there were few degrees of chromatin condensation per unit cell intentionally compared with the cell processed with blood serum non-containing culture medium, it turned out that HBA has controlled the cell death by apoptosis. Since the value was a value near 1.0, it turned out that HBA has the dramatically outstanding depressor effect to the apoptosis by blood serum removal stimulus. It turned out that the apoptotic suppression effect of 80 mmol/L HBA content culture medium has the depressor effect outstanding more slightly than blood serum content culture medium 10%.
[0038]
[Effect of the Invention]In this invention, 3-hydroxybutyric acid and/or its salts are contained as an active principle.
Therefore, the prevention and/or the therapy of the obstacle (except for the obstacle by dry eye) of an eye by apoptosis can be carried out.
the obstacle (except for the obstacle by dry eye) of the eye according to apoptosis by containing the above-mentioned active principle -- prevention and/or the therapy of the obstacle of an angle conjunctiva can be carried out especially.

[Translation done.]